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Structure-based design, synthesis, X-ray studies, and biological evaluation of novel HIV-1 protease inhibitors containing isophthalamide-derived P2-ligands

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ABSTRACT

We describe the design, synthesis and biological evaluation of a series of novel HIV-1 protease inhibitors bearing isophthalamide derivatives as the P2–P3 ligands. We have investigated a range of acyclic and heterocyclic amides as the extended P2–P3 ligands. These inhibitors displayed good to excellent HIV-1 protease inhibitory activity. Also, a number of inhibitors showed very good antiviral activity in MT cells. Compound **5n** has shown an enzyme K_i of 0.17 nM and antiviral IC_{50} of 14 nM. An X-ray crystal structure of inhibitor **5o**-bound to HIV-1 protease was determined at 1.11 Å resolution. This structure revealed important molecular insight into the inhibitor–HIV-1 protease interactions in the active site.

HIV-1 protease inhibitors (PIs) are an important component of highly active antiretroviral therapy (HAART).[1,2] This treatment regimen has had a major impact in improving the quality of life and extending life expectancy of HIV/AIDS patients.[3,4] In fact, recent analyses have revealed that the mortality rates of HIV/AIDS patients have become closer to general mortality rates due to HAART and first-line HAART with protease inhibitor-based regimens.[5,6] Despite these advances, the development of potent and more effective HIV-1 protease inhibitor drugs is essential for successful long-term control of HIV-1 infection and AIDS.[7,8] In our continuing efforts toward the design and synthesis of nonpeptide HIV-1 protease inhibitors with clinical potential, we reported a variety of exceptionally potent PIs including darunavir (**1**, Fig. 1) that incorporated structurally novel ligands and scaffolds targeting the active site HIV-1 protease backbones.[9–12] Our design of ligands has focused on non-peptidic cyclic/heterocyclic structures that mimic the biological mode of peptide bonds. In particular, we designed a variety of cyclic ether and poly-ether derived functionalities inherent to bioactive natural products.[13,14] This led to the development of structurally intriguing bis-THF, Cp-THF, Tp-THF, and Tris-THF-derived exceptionally potent inhibitors with broad-spectrum activity against multidrug-resistant HIV-1 variants.[9–12]

Recently, we reported a series of HIV-1 protease inhibitors incorporating 3-hydroxy-2-alkyl or 3-hydroxy-2-alkoxy benzoic acid derivatives as the P2-ligands.[15,16] A representative example is inhibitor **3**. These inhibitors were designed by taking advantage of the large hydrophobic pocket in the HIV-1 protease S1–S2 subsites.[16] The P2-ligands resemble 3-hydroxy-2-methyl benzamide inherent to nelfinavir (**4**), an FDA approved drug. One of the important features of inhibitor **3** is that the P2 ligand does not contain an asymmetric center. Based upon this result, we have now investigated isophthalamide-based P2-ligands in combination with hydroxyethylamine sulfonamide isosteres. Our preliminary models of such isophthalamide derivatives, based upon the X-ray structure of nelfinavir-bound HIV-1 protease, revealed that both carboxamide functionalities of the isophthalamide ligand can form hydrogen bonds with the Gly-27 backbone carbonyl as well as with Asp-29

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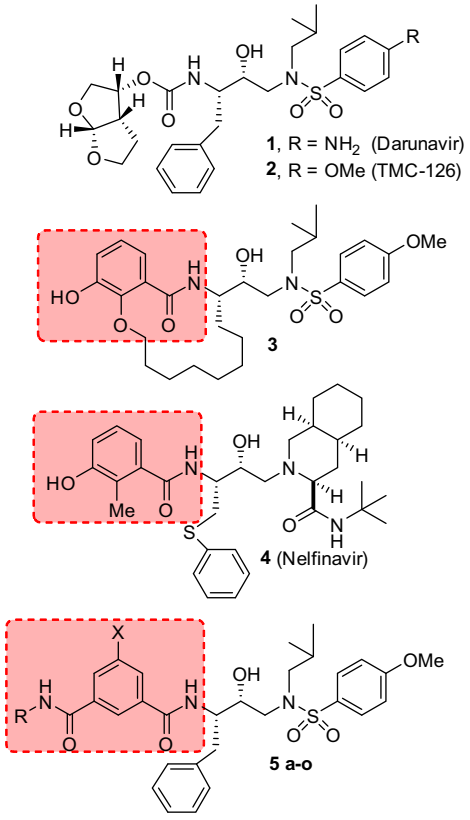
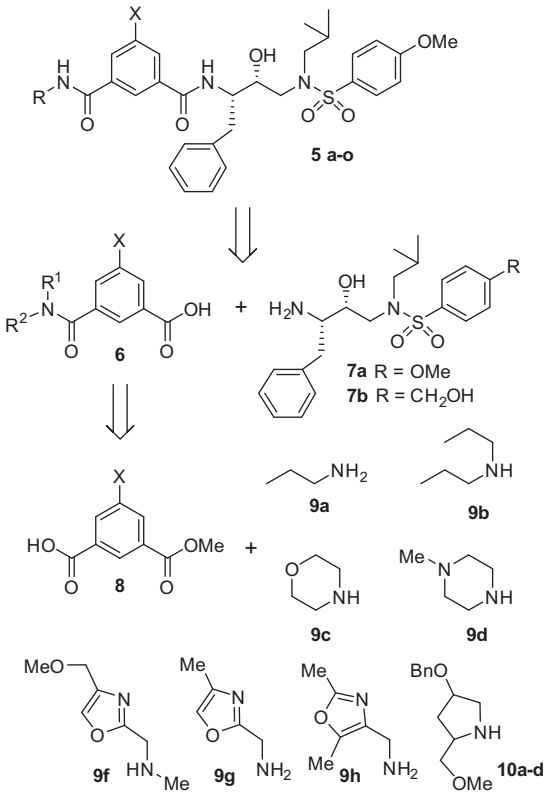


Figure 1. Structures of HIV-1 protease inhibitors **1**–**5**.

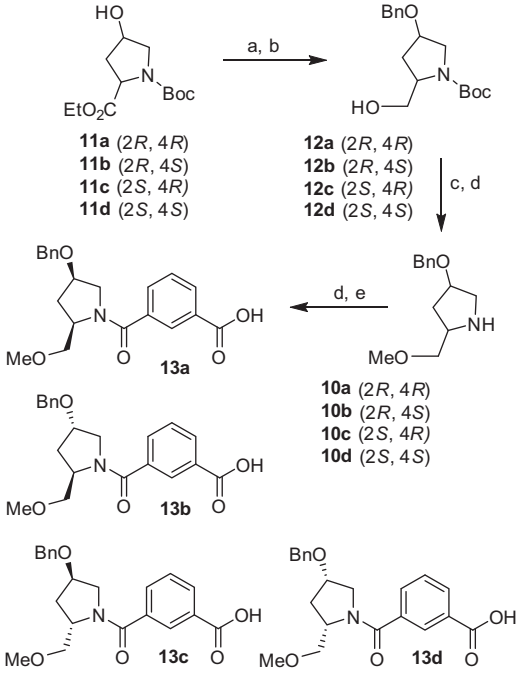


Scheme 1. General synthetic routes to inhibitors.

backbone amide NHs.[17] Also, we speculated that appropriately functionalized substituents can fill in the hydrophobic pocket in the S2–S3 subsites. Interestingly, isophthalamide derivatives were previously utilized in the design of BACE1 inhibitors, an aspartic acid protease implicated in Alzheimer's disease.[18–20] However, there is hardly any report of the use of isophthalamide-derived P2 ligands in the design of HIV-1 protease inhibitors. Herein, we report the design and synthesis of a series of potent HIV-1 protease inhibitors incorporating substituted isophthalamide as the P2–P3 ligands. These inhibitors displayed excellent enzyme inhibitory potency and a number of inhibitors exhibited excellent antiviral activity. A high resolution X-ray structure of an inhibitor-bound HIV-1 protease has been determined. The structure revealed important molecular insight into the ligand-binding site interactions.

Our general strategy for the synthesis of HIV-1 protease inhibitors bearing isophthalamide derivatives as the P2–P3 ligands is shown in Scheme 1. As shown, the designed inhibitors would be synthesized by amide coupling of isophthalic acid derivative **6** with the amine of the hydroxyethylamine sulfonamide isosteres (**7a** and **7b**)[21,22] to provide HIV-1 protease inhibitors. Various isophthalic acid derivatives would be obtained by coupling of readily available isophthalic monoacids **8** with various amines **9a**–**g** and **10**. Among these, amines **9a**–**d** are commercially available. Other amines were synthesized as follows.

The synthesis of various proline derivatives **10a**–**d** is shown in Scheme 2. The synthesis of all four optically active hydroxyl proline derivatives **11** was carried out as described by Baker and co-workers.[23] Protection of the hydroxyl group as the benzyl ether followed by $LiBH_4$ reduction of the ethyl ester provided respective primary alcohol **12a**–**d** in excellent yields. These alcohols were converted to methyl ethers by treatment with NaH in THF for 30 min at 23 °C followed by addition of methyl iodide and stirring for 12 h.



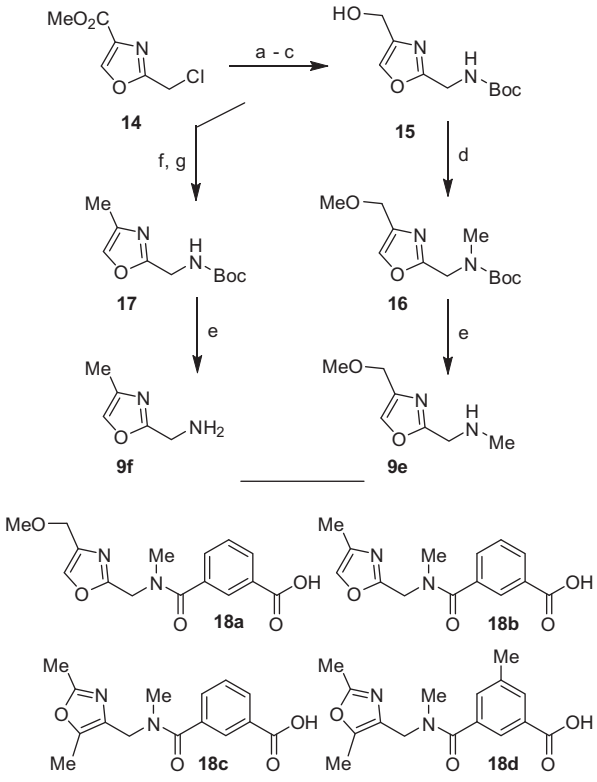
Scheme 2. Reagents and conditions: (a) BnBr, NaH, DMF, 23 °C, 1 h; (b) $LiBH_4$, THF, 23 °C, 2 h (80–85%); (c) MeI, NaH, THF, 23 °C, 12 h; (d) TFA, CH_2Cl_2, 0 °C, 2 h (65–71%); (e) acid **8** (X = H), EDC, HOBT, *i*-Pr_2NEt, CH_2Cl_2, 23 °C; (f) 1 N LiOH, THF, 23 °C, 2 h (67–82%).

Methyl ethers were obtained in good yield. Removal of the Boc-group by exposure of the Boc-derivatives to trifluoroacetic acid (TFA) in CH_2Cl_2 at 0 °C for 2 h afforded proline derivatives **10a**–**d**

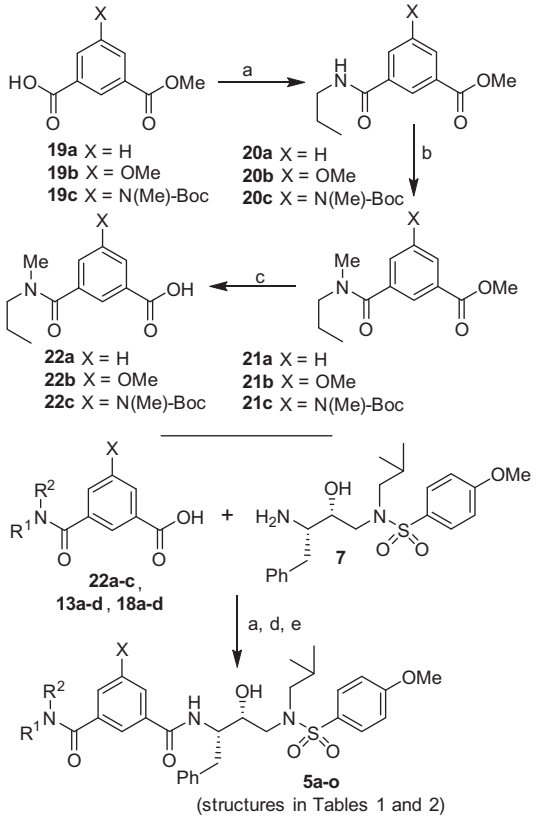
in excellent yields. These proline derivatives were then coupled with isophthalic acid methyl ester **8** (X = H) in the presence of EDC, HOBt, and *i*-Pr_2NEt to provide the corresponding amide derivative. Saponification of the methyl ester with aqueous lithium hydroxide afforded acid derivatives **13a–d** in very good yields.

The synthesis of oxazole derivatives **9e** and **9f** is shown in Scheme 3. Commercially available oxazole **14** was treated with NaN_3 in DMF and heated to 120 °C for 2 h to provide the corresponding azide in 59% yield. Catalytic hydrogenation of azide using 10% Pd–C in the presence of Boc_2O afforded the corresponding Boc-derivative in near quantitative yield. Reduction of the ester with calcium borohydride afforded alcohol **15** in excellent yield.[24] Reaction of alcohol **15** with NaH and MeI in THF for 6 h at 23 °C resulted in the formation of methyl ether and *N*-methylation to provide **16** in moderate yield. Removal of the Boc-group by exposure to TFA in CH_2Cl_2 at 0 °C for 3 h provided amine **9e** in near quantitative yield. Alcohol **15** was converted to methyl oxazole **17** in a two-step sequence involving mesylation of alcohol **15** with mesyl chloride and pyridine at 0–23 °C for 4 h followed by reduction of the resulting mesylate with $NaBH_4$ in HMPA to provide **17**. Removal of the Boc-group by exposure of **17** to TFA in CH_2Cl_2 provided amine **9f** in excellent yield. Amine **9e** was coupled with isophthalic acid methyl ester **8** (X = H) using EDC, HOBt and in the presence of *i*-Pr_2NEt to furnish the corresponding amide derivative. Saponification of the methyl ester with aqueous lithium hydroxide afforded acid **18a** in very good yield. Similarly, acid **8** was coupled with oxazolylmethyl amine **9f** to provide the corresponding amide. The resulting amide was methylated using NaH and MeI in THF for 6 h to provide the corresponding amide. Saponification of the methyl ester with aqueous lithium hydroxide afforded acid **18b**. For the synthesis of dimethyloxazole derivatives **18c**,**d**, the corresponding known oxazolylmethyl amine **9g** was coupled with acids **8** (X = H, Me).[19] The resulting amides were methylated with NaH and MeI to provide the corresponding *N*-methyl derivatives. Saponification of the methyl ester afforded acids **18c** and **18d** (50–55% yield for the 3-steps).

Synthesis of HIV-1 protease inhibitors **5a–o** containing isophthalamide as the P2 ligands is shown in Scheme 4. Coupling of various carboxylic acids **19a–c** with amine **9a** using EDC and HOBt in the presence of *i*-Pr_2NEt afforded the corresponding amide derivative **20a–c** in good yields (50–60%). For the synthesis of *N*-methyl amine derivatives, methyl esters **20a–c** were reacted with NaH and MeI in THF at 0–23 °C for 6 h to provide the corresponding *N*-methyl derivatives **21a–c**. Saponification of the resulting methyl esters with aqueous lithium hydroxide afforded carboxylic acids **22a–c** in good yields (90–95%). For the synthesis of inhibitor **5a**, methyl ester **20a** was saponified and the resulting acid was coupled with amine **7** using EDC and HOBt in the presence of *i*-Pr_2NEt to afford amide **5a** in 65% yield. In the synthesis of inhibitors **5b–d**, amide derivatives **20a–c** were methylated with NaH and MeI to provide the corresponding *N*-methyl amides. Saponification of the methyl ester provided acids **22a–c**. Coupling of acids **22a**,**b** with amine **7** provided inhibitors **5b** and **5c**. Synthesis of inhibitor **5d** was accomplished by coupling of acid **22c** with amine **7** followed by treatment of the resulting amide with TFA in CH_2Cl_2 for 6 h to provide **5d**. For the synthesis of inhibitors **5e–g**, isophthalic acid methyl ester **8** (X = H) was coupled with amines **9b–d**. The resulting esters were saponified and the acids were coupled with amine **7**. For the synthesis of inhibitors **5h–k**, proline derivatives **13a–d** were coupled with amine **7** and the resulting amide derivatives were hydrogenated using 10% Pd–C in a mixture of EtOAc and ethanol. Inhibitors **5l–o** were synthesized by coupling of acids **18a–d** with amine **7a** or **7b**.[21,22]



Scheme 3. Reagents and conditions: (a) NaN_3, DMF, 120 °C; (b) H_2, Pd/C, Boc_2O, EtOAc; (c) $NaBH_4$, $CaCl_2$, EtOH/THF (3:2), (58% 3-steps); (d) MeI, NaH, THF (51%); (e) TFA, CH_2Cl_2, 0 °C (99%); (f) MsCl, pyridine, CH_2Cl_2 (46%); (g) $NaBH_4$, HMPa (66%).



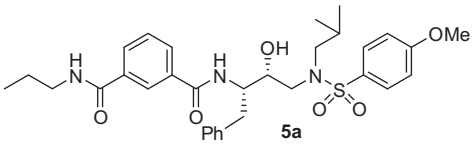
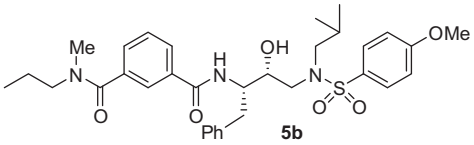
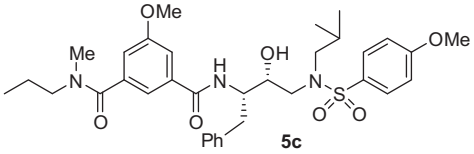
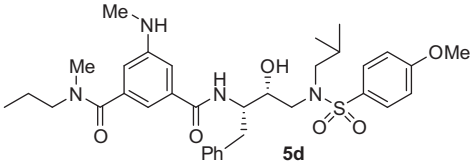
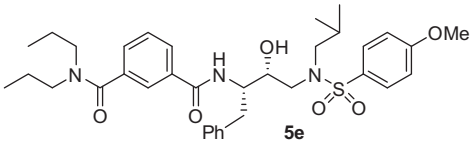
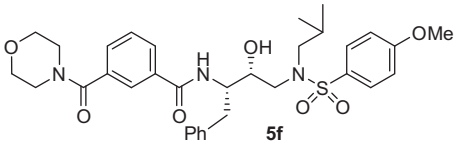
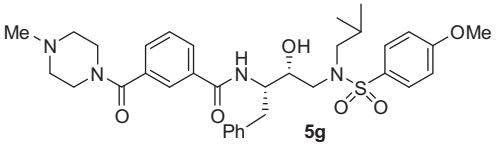
Scheme 4. Reagents and conditions: (a) EDCl, HOBT, DIPEA, CH_2Cl_2; (b) MeI, NaH, DMF; (c) 1 N LiOH, THF, 23 °C, 2 h; (d) TFA, CH_2Cl_2 (90%); (e) H_2, 10% Pd–C, EtOAc/EtOH (1:1), 23 °C, 2 h (90–95%).

Standard EDC and HOBt coupling afforded the various amides in 55–65% yield.

HIV-1 protease inhibitory potency of all inhibitors was first evaluated using assay protocol reported by Toth and Marshall.[25] Compounds that showed potent enzyme inhibitory K_i values were then evaluated in an antiviral assay. The results of isophthalamide-derived inhibitors are shown in Tables 1 and 2. As can be seen, *N*-alkylated amides are more potent than the secondary amide inhibitor **5a** (entry 1). Interestingly, *N*-methyl amide derivative **5b** showed 5-fold enhanced potency over **5a** (entry 2). In inhibitors **5c** and **5d**, we examined the effect of polar substituents at the 3-position of isophthalamide (entries 3 and 4). As it turned out, neither the hydrogen bond donor nor acceptor group improved potency over unsubstituted derivative **5b**. Dipropyl amide **5e** was less effective than *N*-methyl amide **5b**. Compound **5e** was evaluated in antiviral assay using MT-2 cells exposed to HIV-1 LAI.[26] However, it showed no appreciable antiviral activity. Substitution of *N*-methyl-*N*-propyl amide in **5b** with cyclic amines such as morpholine and *N*-methylpiperazine in inhibitors **5f** and **5g** resulted in over 10-fold improvement of protease inhibitory activity (entries 6 and 7). Both inhibitors **5f** and **5g** showed very good antiviral activity as well.

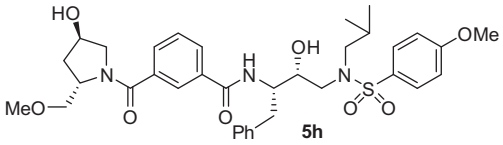
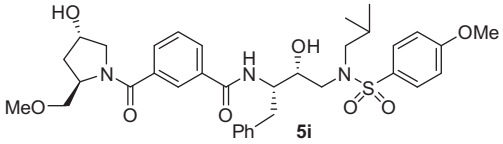
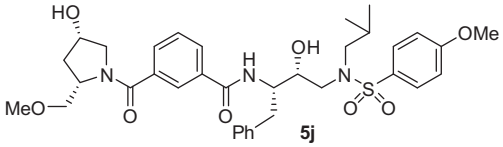
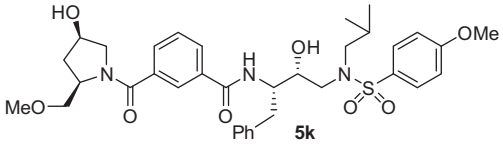
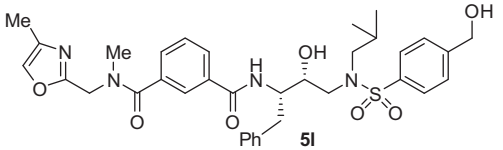
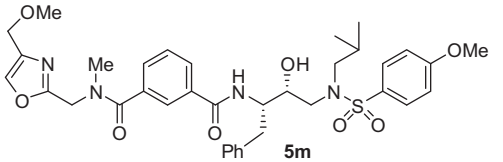
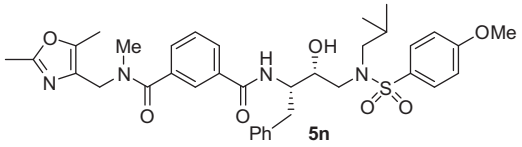
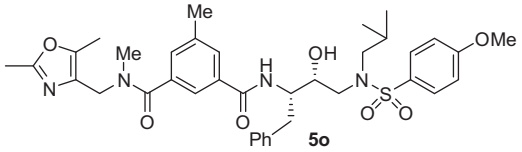
We then examined various proline-derived isophthalamide derivatives. These results are shown in Table 2. Both donor and acceptor functionalities were introduced to form hydrogen bonds in the S2–S3 subsites. We explored all four possible diastereomers of hydroxyproline ligand. As shown, the 2-(*R*)-methoxymethyl configuration is preferred (entries 2 and 4) over 2-(*S*)-diastereomers in inhibitors **5h** and **5j** (entries 1 and 3). The effect of the C-4 hydroxy stereochemistry was not very pronounced. Inhibitors **5h**, **5i** and **5k** showed moderate antiviral activity. We then examined various oxazolylmethyl-derived P3-ligands in inhibitors **5l**–**o**. Inhibitors **5l** and **5m** (entries 5 and 6) exhibited comparable HIV-1 protease inhibitory activity, indicating minor contribution of the methoxy functionality in inhibitor **5m**. Inhibitor **5m** displayed an antiviral IC_{50} value of 25 nM. Inhibitors **5n** and **5o** containing dimethyloxazole derivatives

Table 1
Enzyme inhibitory and antiviral activity of inhibitors **5a–g**

Entry	Inhibitor structure	K_i (nM)	IC_{50}[a,b]
1	 **5a**	25.1	nt
2	 **5b**	5.1	nt
3	 **5c**	20.4	nt
4	 **5d**	157.7	nt
5	 **5e**	12.4	>1000
6	 **5f**	0.42	25
7	 **5g**	0.54	45

[a] Darunavir (**1**) exhibited K_i = 16 pM, antiviral IC_{50} = 3 nM.
[b] nt = not tested.

Table 2
Enzyme inhibitory and antiviral activity of inhibitors **5h–o**

Entry	Inhibitor structure	K_i (nM)	IC_{50}[a,b]
1	 **5h**	2.85	178
2	 **5i**	0.27	389
3	 **5j**	1.5	>1000
4	 **5k**	0.27	190
5	 **5l**	0.63	nt
6	 **5m**	0.31	25
7	 **5n**	0.17	14
8	 **5o**	0.21	31

[a] Darunavir (**1**) exhibited K_i = 16 pM, antiviral IC_{50} = 3 nM.
[b] nt = not tested.

showed enhanced potency over methyloxazole derivative in inhibitor **5l**. However, incorporation of 3-methyl on the isophthalic phenyl ring (entry 8) resulted in reduction of enzyme affinity and antiviral activity. Inhibitor **5n** displayed very potent antiviral activity (IC_{50} = 14 nM). To obtain molecular insight into the inhibitor–HIV-1 protease interactions, we have determined the X-ray crystal structure of the related inhibitor **5o** and HIV-1 protease complex.

The crystal structure of **5o**-bound HIV-1 was refined using X-ray data at 1.11 Å resolution. The overall protease structure showed similarity to that of the complex with darunavir[27] with an RMSD of 0.21 Å for Cα atoms. The largest differences between corresponding Cα atoms are 0.9 Å for residue 78–79, 0.6 Å for flap residues 48 and 51 and 0.9 Å for the N-terminal residue 3′ and 4′, which may result from crystallographic packing.[28] Most of the interactions of the inhibitor with protease residues in the active site cavity are comparable to those of the protease–darunavir complex.[27] The major differences are due to the dimethyl-oxazolylmethyl isophthalamide group in place of the bis-tetrahydrofuranyl (bis-THF) urethane in darunavir. The long P2–P3 ligand is positioned between the side chains of Asp29 and Arg8′ on one side and the carbonyl oxygen of Gly48 on the opposite site, while forming a water-mediated hydrogen bond with the carbonyl oxygen of Pro81′ (not shown in Fig. 2). A new hydrogen bond is formed between the amide nitrogen of Asp29 and one of the carbonyl oxygen of isophthalamide ligand. Also, C–H. . .O interactions are evident between the carbonyl oxygen of Gly48 and amide

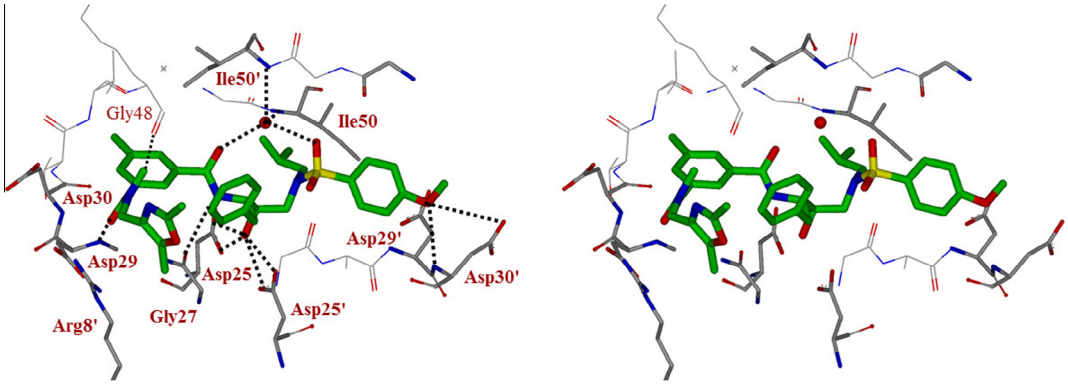


Figure 2. Stereoview of the X-ray structure of inhibitor **5o** (green)-bound HIV-1 protease (PDB code: 4ZIP). All strong active site hydrogen bonding interactions of inhibitor **5o** with HIV-1 protease are shown as dotted lines.

methyl in the P2 ligand, producing a shift in the flap regions 47–56. The five-membered oxazole ring at the distal end of the P2 group rotates into two alternative conformations. The major conformation of the oxazole ring forms hydrophobic interactions with the side chains of Leu23′ and Val82′. The minor conformation of the ring forms C–H...π stacking interactions with the guanidinium moiety of Arg8′. These extended interactions of the long P2 group are expected to stabilize the inhibitor in the binding site. The methylbenzene moiety of the P2 ligand forms C–H. . .π interactions with Ala28 on one side of the binding site and van der Waals contacts with Ile50′, Val32, Ile47 and Ile84 on the other side.

In summary, we have designed and synthesized a series of novel HIV-1 protease inhibitors and evaluated their enzyme inhibitory and antiviral activity. We designed these inhibitors based upon the X-ray structures of inhibitor–HIV-1 protease complexes. The inhibitors incorporated isophthalamide-derived P2–P3 ligands to interact with the HIV-1 protease active site in the S2 and S3 subsites. We have investigated a variety of acyclic, cyclic, and heterocyclic amide derivatives. In particular, we examined substituted prolines and oxazoles which contain hydrogen bond donor and acceptor groups for specific interactions in the S3 subsite. A number of inhibitors exhibited excellent enzyme inhibitory and antiviral activity. Inhibitor **5n** containing isophthalamide dimethyloxazolylmethyl amide displayed an enzyme K_i of 0.14 nM and antiviral IC_{50} value of 14 nM in MT cells. To obtain molecular insight into the inhibitor's binding properties in the HIV-1 protease active site, we determined the X-ray structure of **5o**-bound HIV-1 at 1.11 Å resolution. Our structural analysis revealed that one of the carboxamide NH formed a strong hydrogen bond with Gly27 carbonyl group of HIV-1 protease. The other carbonyl oxygen was involved in hydrogen bonding with Asp 29 NH in the S2 subsite. Further design and improvement of inhibitor properties utilizing this molecular insight are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.05.052.

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28. The optimized HIV-1 protease was expressed and purified as described.[29] The protease–inhibitor complex was crystallized by the hanging drop vapor diffusion method with well solutions of 0.95 M NaCl, 0.1 M Sodium Acetate buffer (pH 5.0). X-ray diffraction data were collected on a single crystal cooling to 90 K at SER-CAT (22-ID beamline), Advanced Photon Source, Argonne National Lab (Chicago, USA) with X-ray wavelength 0.8 Å, and processed by HKL-2000[30] with Rmerge of 6.7%. Using one of previous isomorphous structures as the initial model,[31] the crystal structure was solved by Molrep in CCP4i Suite and refined by SHELX-97 to 1.11 Å resolution.[32,33] PRODRG-2 was used to construct the inhibitor and the restraints for refinement.[34] COOT was used for manual modification of the structure.[35] Alternative conformations were modeled, anisotropic atomic displacement parameters (B factors) were applied for all atoms including solvent molecules. The final refined solvent structure comprised three Na^+ ions, three Cl^- ions, one glycerol and 256 water molecules. The coordinates and structure factors for the structure of HIV-1 protease with inhibitor **5o** have been deposited in Protein Data Bank with code: 4ZIP.
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